

**Supplementary Table 2. Primers' sequence**

*mTOR* 3'UTR 5'  
GGCTCGAGAGATGTGCCCATCACGTT

*mTOR* 3'UTR 3'  
GGCGGGCCGCTGATGTCATTATTGGCACA

*raptor* 3'UTR 5'  
GGCTCGAGCCTGCTACTCGCTTTGTC

*raptor* 3'UTR 3'  
GGCGGGCCGCTTCCGAATTCCAGTGTC

*IGF-1R* 3'UTR 5'  
GGCTCGAGTCTCGGAGTTAAGGCGAATTG

*IGF-1R* 3'UTR 3'  
GGCGGGCCGCTATTCTGTTCTACAAAAATGCAATG

**Supplementary Table 3. Quantification of phospho-mTOR (Ser2448) and phospho-RPS6 (Ser240/244) staining in ACT**

<i>Tumor #</i>	<i>phospho-mTOR staining</i>	<i>phospho-RPS6 staining</i>
ACT30	+++	+++
ACT31	++	++
ACT32	+++	++
ACT33	+	++
ACT34	++	++
ACT35	++	++

0, negative; +, weak staining; ++, intermediate staining; +++, strong staining.

## **Legends to Supplementary Figures**

**Supplementary Figure 1.** Taqman RT – qPCR expression analysis of miRNAs found differentially expressed in childhood ACT by microarray (black histograms), plus the let-7a miRNA as a control (white histogram). Data are plotted as ratios to normal tissue expression, which was set as equal to 1.

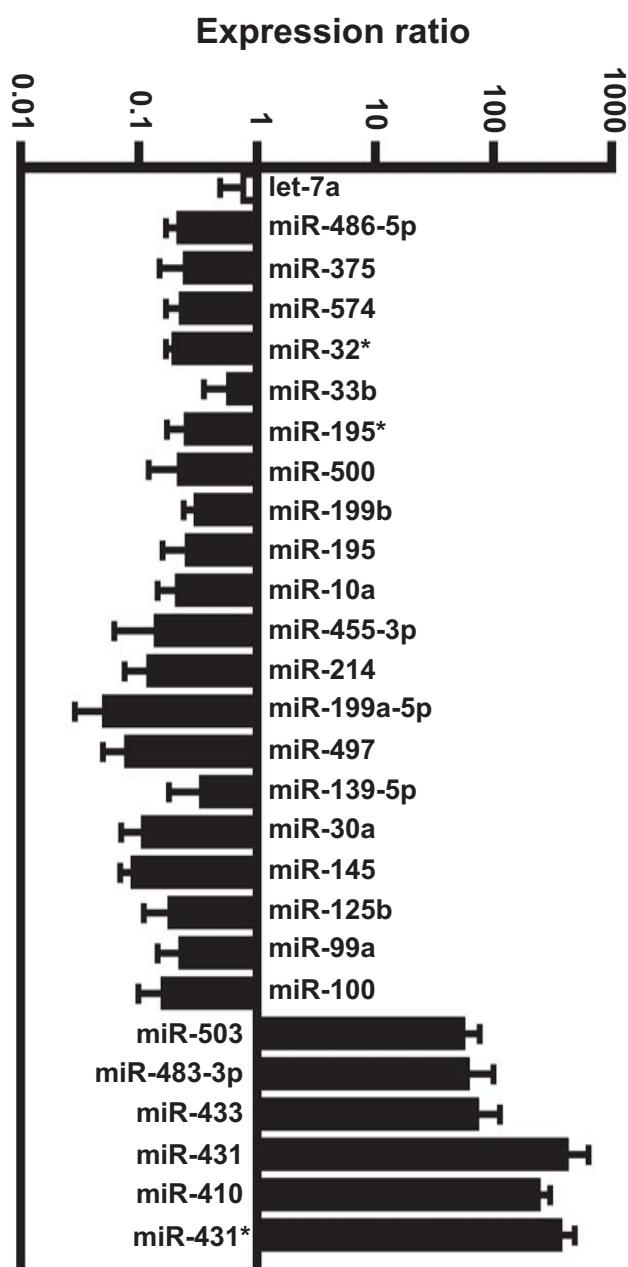
**Supplementary Figure 2.** Conservation of miR-99a/miR-100 binding sites in mTOR (FRAP1) (A) , raptor (RPTOR) (B) and IGF-1R (IGF1R) (C) 3' UTRs.

**Supplementary Figure 3.** Quantification of mTOR (A), phospho-mTOR (Ser2448) (B), raptor (C) and IGF-1R (D) protein expression in ACT and normal adrenal samples. \*\* p<0.01, Student's *t*-test.

**Supplementary Figure 4.** Distribution of total mTOR (upper panels) and Ser2448-phosphorylated mTOR (lower panels) in H295R cells. 100x magnification, bar = 10  $\mu$ m.

**Supplementary Figure 5.** Inhibition of mTOR signalling by RAD001 in H295R xenografts. **A)** Tumor weight in animals treated with placebo (white histogram) and RAD001 (10 mg/kg/day; black histogram). \* p<0.05, Student's *t*-test. **B)** Number of mitoses per high power microscopic field (x40) in xenografts from animals treated with placebo (white histogram) and RAD001 (10 mg/kg/day; black histogram). \*\* p<0.01, Student's *t*-test. **C)** Top panels: Hematoxylin-eosin staining of xenografts from animals treated with placebo (left) and with RAD001 (right). Note the presence of a thrombus (arrowhead) inside a blood vessel in RAD001-treated xenografts. Middle panels: Blood vessels staining by Texas red-conjugated tomato lectin. Bottom panels: Phospho-RPS6 immunoreactivity. 40x magnification, bar = 20  $\mu$ m.

Suppl. Figure 1



## Suppl. Figure 2

A

FRAP1

	290	300	
<i>H. sapiens</i>	UAGAAA	<b><i>UACGGGU</i></b>	UUUGAC
<i>M. musculus</i>	GAGAAA	<b><i>UACGGGU</i></b>	UUUGAC
<i>O. anatinus</i>	GAGAAA	<b><i>UACGGGU</i></b>	UUGUCG
<i>G. gallus</i>	ACUGUA	<b><i>UACGGGU</i></b>	UUGGGC

B

RPTOR

	400	410	
<i>H. sapiens</i>	GGCUCC	<b><i>UACGGGU</i></b>	CCCUGG
<i>P. troglodytes</i>	GGCUCC	<b><i>UACGGGU</i></b>	CCCUGG

C

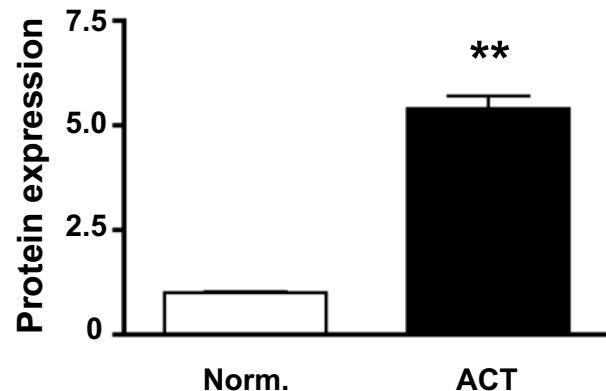
IGF1R

	5590	5600	
<i>H. sapiens</i>	GUAGAU	<b><i>UACGGGU</i></b>	GUCAGU
<i>M. musculus</i>	GUAGAU	<b><i>UACGGGU</i></b>	GUCGUU
<i>O. anatinus</i>	GUAGAU	<b><i>UACGGGU</i></b>	GUCAGU
<i>G. gallus</i>	GUAGAU	<b><i>UACGGGU</i></b>	GUCAGU

Suppl. Figure 3

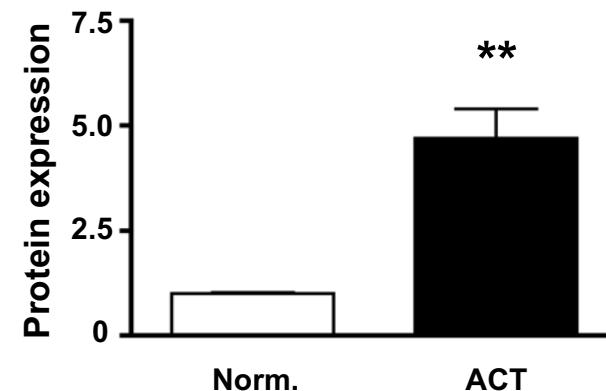
A

mTOR



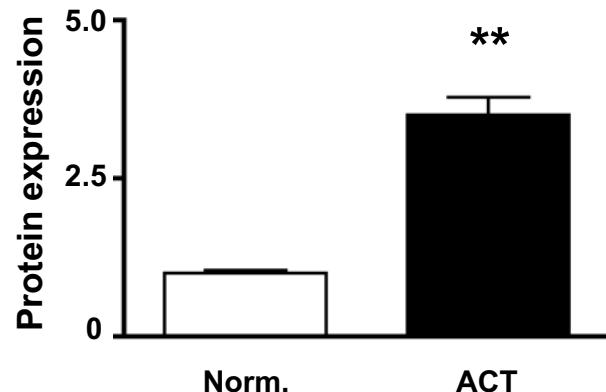
B

S2448 p-mTOR



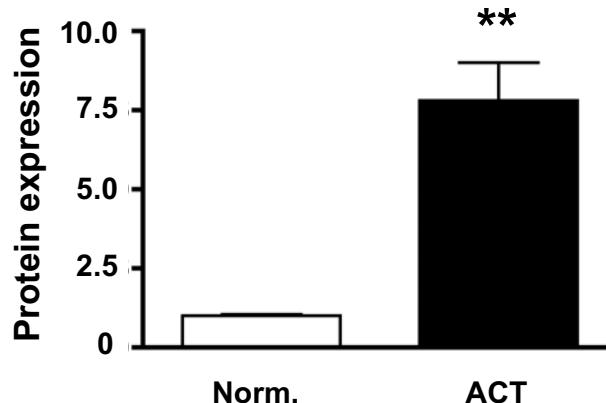
C

raptor

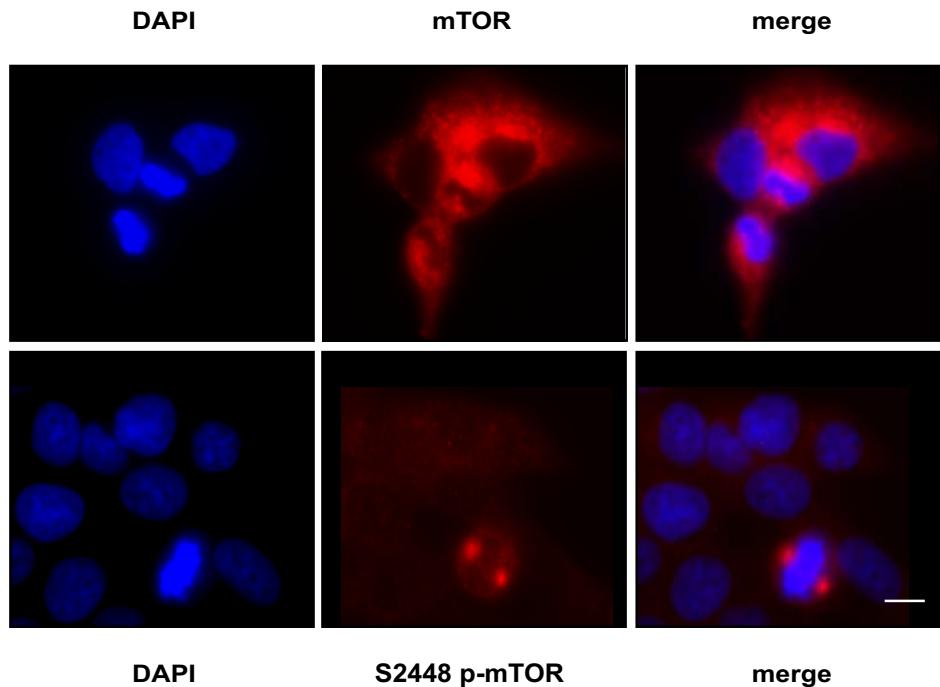


D

IGF-1R

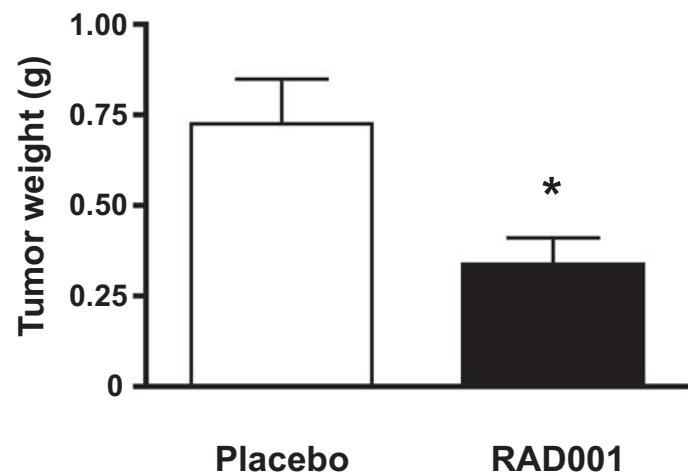


**Suppl. Figure 4**

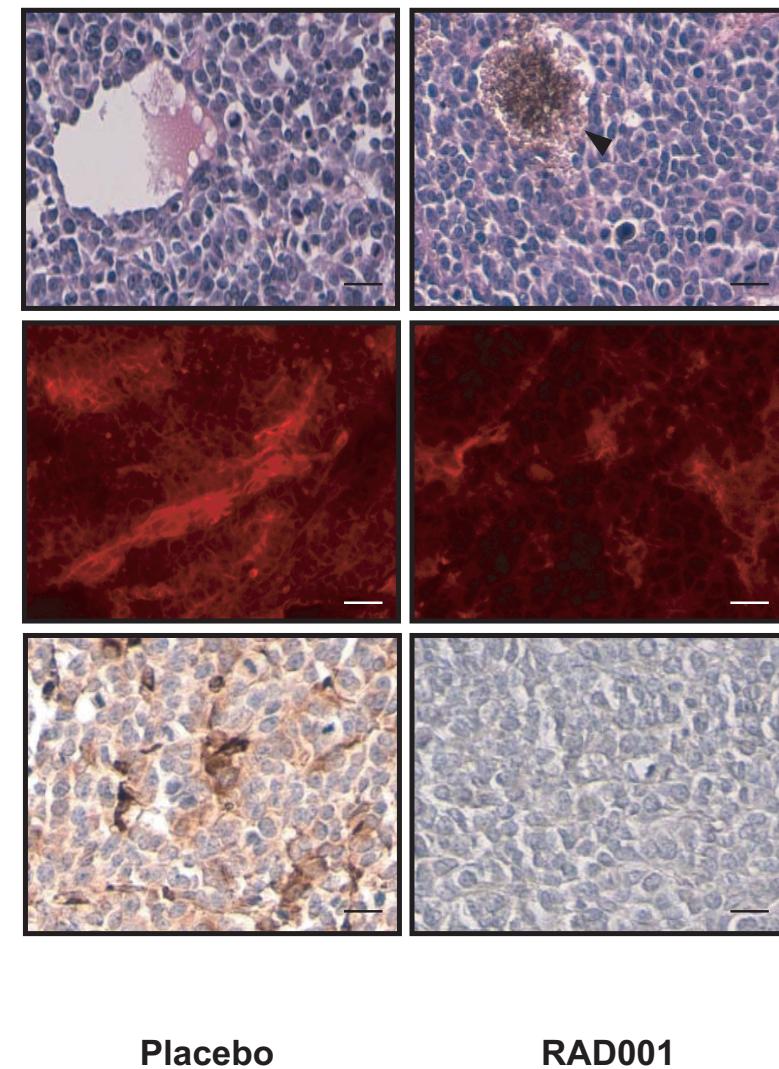


Suppl. Figure 5

A



C



B

